Intrinsically Disordered Proteins II

2428-Pos Board B120
MD Simulations of Intrinsically Disordered Proteins with Replica-Averaged Chemical Shift Restraints
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Molecular dynamics simulations represent a powerful method for exploring the conformational space of folded proteins. However, the success has so far been limited when the method is applied to intrinsically disordered proteins, a situation that can be attributed to force field inaccuracy and sampling inefficiency. To address the issue, we have developed a strategy to combine the chemical shift information with molecular dynamics simulations for characterizing the structural ensembles corresponding to intrinsically disordered proteins. This method is based on the CamShift protocol for calculating the chemical shifts from inter-atomic distances and to calculate forces that minimize the deviations between experimental and calculated chemical shifts. We have used chemical shifts as these NMR parameters are most convenient for the study of intrinsically disordered proteins, since they, at least in principle, contain information about the structure and dynamics of the molecules. To further enhance the sampling efficiency, the method of metadynamics approach with replica exchange is added to the protocol. The capability of the protocol is demonstrated with in the case of the fragment F4 of tau (tauF4 = tau[Ser208-Ser324]).

2429-Pos Board B121
Molecular Dynamics Studies of Tau Monomer and Dimer Conformations
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The microtubule associated protein tau is important in nucleating and maintain microtubule spacing and structure in neuronal axons. Over-phosphorylated tau is implicated in Alzheimer's disease, where it is found in plaques. Tau likely forms dimers as there is only one microtubule binding domain per tau. Because tau in an intrinsically disordered protein, conventional modeling techniques are not sufficient to accurately describe its structure. In order to simulate tau, we therefore must generate many starting structures in order to form a more complete ensemble. We present preliminary molecular dynamics studies for the n-terminal half of the tau monomer and dimer models (residues 1-241) for tau's normal function as a microtubule spacer and adduce the distributions of end-to-end separations. Because normal tau has several phosphorylation sites, we also investigate how varying phosphorylation locations on the n-terminal half of tau affect its structure.
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2430-Pos Board B122
Molecular Mechanism of Interfacial Adsorption of Disordered Cytoplasmic Tail of Immune Receptors to Membrane
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Important immune responses are linked to Src-mediated phosphorylation of cytoplasmic tyrosine-based motifs (ITAMs). However, the mechanism of how receptor ligation translates into ITAM phosphorylation remains elusive. Here, we use molecular dynamic simulations to explore the potential regulatory involvement of lipid membranes and their influence on the structure and behavior of the cytoplasmic portion of the CD3ε chain of the T-cell receptor. It has been hypothesized that the accessibility of ITAM motifs, such as those in the CD3ε cytoplasmic tail, can be blocked by ionic interactions between positively charged amino acids in receptor tails and negatively charged lipid head groups. This interesting hypothesis represents a previously unrecognized mechanism for control of receptor activation. Our simulations support the notion that the net charge of the lipids present in the membrane can affect peptide-membrane interactions. Results are consistent with experimental findings that show increase interfacial absorption in negatively charged lipid bilayer. Our simulations revealed the conformational variability of the disordered tail, which led to an additional focus on quantifying the interaction by free energy calculations, combined with long time-scale simulations using coarse-grained (CG) approaches. These studies will be extended to address how changes in ionic conditions can modulate phosphorylation of ITAM motifs and lead to regulation of activation of the TCR and other ITAM-bearing immunoreceptors.

2431-Pos Board B123
The Effect of Proline CIS Trans Isomerization on P53 MDM2 Binding
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Proline is unique in that it is the only amino acid that adopts both cis and trans conformations in proteins. In spite of the importance of proline isomerization as a molecular switch in proteins, the effect on protein binding has not been thoroughly investigated, especially for intrinsically disordered proteins (IDPs). In this study, a potential of mean force method was used to calculate the absolute binding affinities for the disordered p53 and MDM2 when the proline in p53 is in both cis and trans conformations. To obtain converged affinity results it was necessary to apply conformational, axial, and orientational restraints to the protein internal coordinates. Our results give insight into how isomerization of a proline affects binding of an IDP to a structured protein.

2432-Pos Board B124
Coarse Grain Models Highlight the Importance of Flexible Disordered Linkers as Determinants of the Phase Behavior in Polyvalent Proteins
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Micron-sized, non-membrane bound cellular bodies can form as the result of collective, heterotypic interactions. These bodies form as the result of micron-scale phase separation akin to liquid-liquid demixing, microscale gelation, or a convolution of these processes. Recent efforts have focused on various biophysical aspects of the phase behavior of macromolecules. This interest is catalyzed by the recognized functional importance of various cellular bodies that result from micron-scale phase transitions.
Li et al. have quantified the effects of polyvalent interactions between macromolecules. For the case of binary interactions between a polymer of SH3 domains and a polymer of proline-rich modules (PRMs), valence refers to the numbers of SH3 domains and PRMs within the respective polymers. Within each polymer, flexible linkers connect the interacting units (SH3 domains and PRMs). Here, we probe the effects of linker properties including lengths, flexibility, and asymmetry of properties between the proteins as determinants of the concentration dependence of phase transitions. Our approach utilizes a lattice-based model that belongs to the same universality class as a three-dimensional Ising model.
We find that that the critical concentration for phase transition is inversely correlated with both the lengths and random-coil-like character of intrinsically disordered linkers. Our observations imply the entropic penalty associated with loop closure promotes the growth of a network. We also show, in consistency with published experiments, that asymmetry in linker lengths of the interacting proteins will manifest itself as asymmetries in details of the concentration dependencies within the phase diagrams of two-component systems. Our results provide a physical rationale for selection against compact disordered linkers that connect interacting modules.
1. Li, P. et al., (2012) Nature, 483: 336-340.

2433-Pos Board B125
Computational Characterization of the Disordered Ensembles of Vasopressin and Oxytocin
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Vasopressin and oxytocin are intrinsically disordered cyclic nanopeptides belonging to a family of neurohypophysial hormones. Vasopressin is an antidiuretic, regulating the retention of water and salts in mammals by indirectly promoting the insertion of aquaporin-2 channels into the epithelial cells of kidney nephron collecting ducts. Oxytocin, in contrast, is a neurotransmitter responsible for inducing labor and the subsequent lactation, as well as modulating some social behaviors. Although unique in their functions, these peptides only differ by two residues and both feature a tocin ring formed by the disulfide bridge between 1st and 6th cysteine residues. This structural similarity was experimentally linked to inhibition of activity at vasopressin receptors by the present oxytocin. Conversely, previous studies have also shown that single-residue mutations in both peptides have a significant impact on their receptor specificities.
In this study we perform molecular dynamics (MD) simulations of wild type and mutant oxytocin and vasopressin in order to characterize their structural

ensembles and relate them to the observed variation in activity. The generated ensembles of Y21H and P26L mutants of vasopressin and Q23T and Q23T,P26G mutants of oxytocin reveal significant population shifts compared to the wild type peptides. Here we present the classification of these populations based on the distribution of radius of gyration and deformation of the tocin ring, and attempt to relate the structural changes to the experimentally determined peptide activity and carrier protein binding affinity.

2434-Pos Board B126

Understanding the Evolution of Dynamics for an Intrinsically Disordered Protein

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It is not fully understood how the evolution of intrinsically disordered proteins (IDPs) differs from that of ordered proteins. It is currently thought that IDPs are capable of performing diverse roles in the body due in part to their dynamic nature. The formation of transient secondary structures by IDPs allows them to explore their environment and interact promiscuously with several ordered proteins. To explore the role of dynamics in IDP evolution we used our broad ensemble generation with reweighting method to generate ensembles of structures for the disordered protein p53 and naturally occurring mutants. We then used anisotropic network modeling to quantify the dynamics of these proteins and found a reduction in the root-mean-squared fluctuations for mutant structures compared to wildtype. Our results suggest that IDPs are under selective pressure to maintain high levels of structural dynamics.

2435-Pos Board B127

The Mechanism of Amyloid-β42 Fibril Elongation

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Amyloid-β, an intrinsically disordered protein, forms amyloid fibrils in the brains of patients with Alzheimer's disease. A recent study suggests that fibrils can catalyze the formation of neurotoxic soluble oligomeric species. We therefore explored the folding free energy landscape of Amyloid-β42 using extensive molecular dynamics (>50 μs) coupled with umbrella sampling. We find that fibril elongation occurs through a funnel-shaped, downhill free energy pathway, and we identify a key on-pathway intermediate that involves the interaction of a beta hairpin with the fibril core. Furthermore, an analysis of the minimum-energy pathway suggests two potential mechanisms by which fibrils can act as catalysts for soluble oligomer formation: 1) pre-existing fibrils provide a nucleation site at which the local concentration of monomers is increased and; 2) fibril elongation involves monomeric intermediates that can be incorporated into soluble oligomers. These data provide the first detailed mechanistic description of the elongation of amyloid β fibrils and provide a link between the disordered free monomeric protein on the one hand and the folded fibrillar state on the other. In addition, they suggest a rational drug design strategy that aims to stabilize the fibril-associated hairpin conformation in order to prevent elongation of pre-existing fibrils.

2436-Pos Board B128

Differences in Dynamics and Stability of the Wild Type Beta-Amyloid Aβ1-40, and ΔE22-Aβ1-39 (Japanese) Mutant Protofibril Structures, a Molecular Dynamics Study

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Alzheimer's disease has been identified as a neurodegenerative disorder associated with protein misfolding due to the aggregation of monomeric beta-amyloid proteins (Aβ) to form fibrillar plaques. Experimental attempts to chemically analyze the structure of Aβ protofibrils and elucidate the mechanism of fibril formation have yet to reveal much about the molecular etiology of AD, due to the low solubility and non-crystalline nature of Aβ. It has been shown experimentally that the ΔE22-Aβ1-39 (Japanese) mutation of β-amyloid leads to production of typical Aβ fibrils essentially instantaneously, much faster than the fibril formation in wild-type (WT) Aβ1-40. To better understand the fibril-forming mechanism of the Japanese mutant peptide, we ran several long all-atom explicit water molecular dynamics simulations of the mutant and WT structures starting from NMR Alzheimer's β-amyloid fibrils (PDB-ID: 2LMN, 2LMO, 2LMP, 2LMQ). The NMR data suggest two different configurations of the fibrils consisting of either two- or three-stacks. Our WT simulations show that the two-stack model is energetically the most stable configuration; both stacks twist around their central axis in a cooperative manner to form a helical structure without any separation between strands. In contrast, monomers rapidly separate from the top and bottom of the three-stack systems. Simulations with just one-stack from either the two- or three-stack systems display the same twist motion, but monomers from the top and bottom of the stack start to separate. These observations suggest that the two-stack system is the most stable nucleation unit. Japanese mutants built for the one-stack system starting from WT protofibril models remain more stable (~150 kcal/mol) throughout our simulations compared to the WT one-stack system. This might explain the almost instantaneous fibril formation observed for the ΔE22-Aβ1-39 mutation.

2437-Pos Board B129

Models of Length Dependent Behavior in Polyglutamine based on Improved Simulation Methods

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We present a study of the length-dependent statistical mechanical behavior of monomeric polyglutamine tracts over a broad range of repeat lengths, using implicit solvent as well as novel replica exchange based explicit solvent molecular dynamics techniques that allow for drastic conformational change in peptides of these sizes. First, these methodological changes were validated by comparison with conventional simulations, then equilibrium thermodynamic models were fit to simulation data, showing a crossover from alpha helical structure to either beta sheet rich or disordered structures with increasing monomer length, depending on the choice of solvent model.

2438-Pos Board B130

Influence of Desolvation Barriers in Coupled Folding and Binding Kinetics of pKID-KIX

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Flexible and dynamic encounter complexes associated with coupled folding and binding can promote rapid molecular recognition of intrinsically disordered proteins (IDPs). Weak non-specific interactions between an IDP and its binding partner encourage enhanced binding rates through fly-casting, reduced orientational entropic costs, and long-lived encounter complexes associated with flexible binding partners. Here, we use coarse-grained native-centric simulations to investigate how desolvation barriers between the binding partners influence coupled folding binding kinetics of an IDP (pKID) to its target (KIX). We compare the free energy binding surface and kinetics for models with and without desolvation barriers between pKID and KIX for a range of flexibilities. We find that desolvation barriers leads to enhanced cooperativity of the coupled folding and binding over models without desolvation. Furthermore, when desolvation barriers are present, the simulated binding rate is over an order of magnitude faster when pKID is unfolded in the unbound. The sensitivity in binding rate to the flexibility of pKID can be understood, in part, by the ability of a flexible binding partner to overcome desolvation barriers locally while rigid proteins must overcome them collectively.

2439-Pos Board B131

Different Roles of Backbone-Originated and Native-Contact-Originated Flexibilities of an Intrinsically Disordered Protein in Fast Binding and Unbinding

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The intrinsically disordered protein (IDP) in the unbound state is so flexible that it does not adopt a unique structure. IDP may exploit this flexibility to function as a signaling molecule, switching on and off the signal by binding to and unbinding from the target molecule, considering that the binding/unbinding kinetics is largely affected by the flexibility. For example, it has been suggested that the flexibility enhances the binding rate by the fly-casting mechanism. However, some counter evidences have been reported, so the role of the flexibility on the fly-casting mechanism is still controversial. To address this problem, we examined the effect of two different types of flexibilities that were computationally imparted to IDP to which the one-bead (Cα) Gō-like model was applied; one is by softening the backbone rigidity and the other is by weakening the native contact. We used pKID (kinase inducible domain of CREB) as a model of IDP. pKID folds upon binding to the partner protein, KIX. We calculated the potential of mean force as a function of distance between pKID and KIX. We found that the backbone-originated flexibility and the native-contact-originated one exhibit different results. The native-contact-originated flexibility yielded larger capturing radius and faster binding, consistent with the fly-casting, but the backbone-originated one did not. The strength of the interaction between IDP and the target was also shown to play a critical role. As for unbinding, the backbone-originated flexibility destabilized the bound state more effectively, leading to faster unbinding. Therefore,